Carbohydrate Polymers 108 (2014) 153–158

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

A new method for determining the relative crystallinity of chickpea starch by Fourier-transform infrared spectroscopy

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ARTICLE INFO

Article history:
Received 24 December 2013
Received in revised form 10 February 2014
Accepted 28 February 2014
Available online 12 March 2014

Keywords:
Fourier-transform infrared spectroscopy
X-ray diffraction
Chickpea
Starch
Relative crystallinity

ABSTRACT

A new method for determining the relative crystallinity (RC) of chickpea starch was developed by using Fourier-transform infrared (FT-IR) spectroscopy, based on hypotheses as described as follows: there is a Gaussian holocrystalline-peak (HCP) in the 800–1300 cm^{-1} region of FT-IR spectrum of starch which is divided into amorphous region and crystalline region; the crystalline region of HCP is the overlap of the HCP and the FT-IR spectrum of starch; the RC of starch is the ratio of the area of crystalline region to the area of HCP. It was found that there was no significant difference between the RC determined by FT-IR method and that determined by X-ray diffraction (XRD) method. The intra-class correlation coefficient was 0.998 ($p = 0.000$, $n = 9$) and the 95% confidence interval was 0.992–1.000 for the RC determined by XRD and FT-IR. Furthermore, the developed method showed good repeatability (coefficient of variation (CV), 1.1–2.9%) and good intermediate precision (CV, 2.8%).

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third important pulse crop in the world and rich in starch (37.5–50.8%) and protein (Chavan, Kadam, & Salunkhe, 1986; Jukanti, Gaur, Gowda, & Chibbar, 2012; Saini & Knights, 1984). Based on seed morphology and cultivation area, chickpea can be grouped into two cultivated varieties: Desi and Kabuli. The Desi chickpea has small and dark seeds and a thick coat, cultivated mostly in Asia and Africa; the Kabuli chickpea has large and light colour seeds and a thin and smooth coat, cultivated mostly in Europe, North America, West Asia and North Africa (Chavan et al., 1986; Jukanti et al., 2012).

The starch granules are semi-crystalline with various crystalline structures and degrees of crystallinity which have important impacts on the physical and functional properties of starch such as gelatinization and glycemic response (Cai & Wei, 2012; Parada & Aguilera, 2012; Tester, Karkalas, & Qi, 2004; Yang, Gu, & Hemar, 2013). Based on the difference of X-ray scattering in the crystalline and amorphous regions of starch granule, X-ray diffraction (XRD) is generally used to determine the starch crystallinity (Beninca et al., 2013; Mutungi, Passauer, Onyango, Jaros, & Rohm, 2012; Primo-Martin, van Nieuwenhuijzen, Hamer, & van Vliet, 2007). According to the pattern, the crystalline structures of starches can be classified into A-, B- and C-type crystals (Tester et al., 2004). Nevertheless, it requires expensive equipment and under the best of circumstances. Despite differential scanning calorimetry (DSC) can also be used to estimate the starch crystallinity through measuring the enthalpy of starch in different states, it is not precise due to the crystallization effect during the heating and the variation of heat capacity with temperature (Kong & Hay, 2002; Primo-Martin et al., 2007; Vippagunta, Brittain, & Grant, 2001). Besides, solid-state ^{13}C cross-polarization and magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) is alternative to determine starch crystallinity through the analysis of molecular (double-helical) order, but it is not widely used due to its expense and long analytic time (Cooke & Gidley, 1992; Mutungi et al., 2012).

Fourier-transform infrared (FT-IR) spectroscopy can provide useful information on crystallinity of various materials, such as poly(ethylene terephthalate) and cellulose (Chen, Hay, & Jenkins, 2012; Kljun et al., 2011; Nelson & O'Connor, 1964). This method has been advocated since it is simple, rapid, non-destructive and without complicated sample pretreatment before measurement. In theory, FT-IR can be applied to determine material crystallinity if there is a probe band (varying with material crystallinity) and a

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http://dx.doi.org/10.1016/j.carbpol.2014.02.093
0144-8617/

reference band (providing a kind of internal standard to eliminate the errors produced from sources such as the uncertainty in the sample dosage in KBr disc and the uncontrollable light scattering). Accordingly, starch crystallinity can also be determined by FT-IR if there are a suitable probe band and a suitable reference band in FT-IR spectra. At present, some absorption bands in the 1300–800 cm^{-1} regions of FT-IR spectra, for example the 1047 cm^{-1} and 1022 cm^{-1} bands, have been reported to be related with the crystalline phase and amorphous phase within the starch granules (Ambigaipalan, Hoover, Donner, & Liu, 2014; Ispas-Szabo, Ravenelle, Hassan, Preda, & Mateescu, 2000; Sevenou, Hill, Farhat, & Mitchell, 2002; vanSoest, Tournois, deWit, & Vliegenthart, 1995). However, to our knowledge, there is no independent method to determine starch crystallinity by FT-IR. Therefore, a new independent method to determine the relative crystallinity (RC) of starch by FT-IR was developed in the present study. In order to develop a novel method for the determination of RC of starch by FT-IR, native and resistant chickpea starch samples with different RC were prepared from Kabuli chickpea seeds and new FT-IR spectral bands were selected as a probe band and a reference band.

2. Material and methods

2.1. Materials and reagents

Chickpea seeds were provided by Xinjiang Agricultural University (Xinjiang, China). Sweet potato starch, corn starch and potato starch were purchased from Shandong Jincheng Co., Ltd. (Shandong, China). Wheat starch and tapioca starch were purchased from Shanghai Heyu Trade Co., Ltd. (Shanghai, China). Amyloglucosidase (100 U/mg) was purchased from Shanghai Kayon Biological Technology Co., Ltd. (Shanghai, China) and heat-stable-amylase (20000 U/mL) was purchased from Jiangsu Ruiyang Biotech Co., Ltd. (Jiangsu, China).

2.2. Preparation of chickpea starch samples

2.2.1. Preparation of native chickpea starch (NS)

Chickpea seeds were steeped in water containing 0.3% sodium sulfite for 24 h at room temperature. The grains were then ground by a laboratory blender, and the resulting ground slurry was screened through nylon cloth (100 mesh) and was allowed to stand for 5 h. The supernatant was removed by suction and the settled starch layer was resuspended in distilled water containing 0.04% sodium hydrate to remove protein and further washed by successive static precipitation in distilled water until neutrality. Most part of them was dried as the material for the production of resistant starch in an oven at 70 °C, and the rest was lyophilized as NS.

2.2.2. Preparation of resistant chickpea starch I (RSI)

The preparation of RSI was performed according to the method (Sievert & Pomeranz, 1989) with slightly modifications. Briefly, the chickpea starch was suspended in distilled water (starch/water ratio, 3:10), the resulting suspension was autoclaved at 121 °C for 30 min, cooled to 4 °C and stored at this temperature for 24 h. These autoclaving-cooling cycles were repeated up to three times. Then, the resulting sample was broken up and incubated with a heat-stable α-amylase (500 U/g starch) at 96 °C for 30 min (pH 6.0) and an amyloglucosidase (500 U/g starch) at 59 °C for 30 min (pH 4.5), respectively. The insoluble residues were washed with distilled water by centrifugation, and most part of them was used for further digestion with amylase in excess, the rest was lyophilized as RSI.

2.2.3. Preparation of resistant chickpea starch II (RSII)

RSI was resuspended in an equal volume of distilled water and then incubated with a heat-stable-amylase (5000 U/g wet basis) at 96 °C for 24 h (pH 6.0) and an amyloglucosidase (500 U/g wet basis) at 59 °C for 30 min (pH 4.5), respectively. The insoluble residues were washed with distilled water by centrifugation, and most part of them was used for further treatment in high concentration of K_2CO_3 solution, the rest was lyophilized as RSII.

2.2.4. Preparation of resistant chickpea starch III (RSIII)

RSII was suspended in high concentration of K_2CO_3 solution (ratio of K_2CO_3/water, 4:5). The floated matter was removed and the settled starch was washed by successive centrifugation in distilled water until neutrality and lyophilized as RSIII.

2.3. XRD analysis

XRD analysis was carried out on a Bruker D8 Advance Diffractometer. The radiation used was Cu-Kα (wavelength of 0.15406 nm). The scan was performed at 40 kV and 40 mA for a 2θ range of 5–37°, with a step size of 0.02° and a collection time of 6.7 s at each step. The RC of the starch was quantitatively estimated following the method (Nara & Komiya, 1983) by using MDI Jade 5.0 software (Materials Data Inc., Livermore, CA). All analyses were performed in triplicate.

2.4. FT-IR spectroscopic analysis

The FT-IR spectra of starch samples in KBr discs (2 mg starch/150 mg KBr disc) were recorded using a Nicolet IR200 spectrophotometer with a resolution of 4 cm^{-1} in a spectral range of 4000–400 cm^{-1}. Omnic 8.0 software (Thermo Fisher Scientific Inc., WI, USA) was used for baseline-correction of the FT-IR spectra of starch samples, and Origin 8.0 (OriginLab Corp., MA, USA) was used for fitting the experimental curve. All analyses were performed in triplicate.

2.5. Method validation

The developed FT-IR method was validated following the assay procedures of the International Conference on Harmonisation (ICH) guidelines (ICH Harmonized Tripartite Guideline, 2005). Briefly, linearity, accuracy and specificity were determined by analyzing nine starch samples of NS, RSI, RSII, RSIII, sweet potato starch, corn starch, potato starch, wheat starch and tapioca starch with triplicate measurements. Repeatability was assessed from the results of triplicate determinations of NS, RSI and RSII. Intermediate precision was estimated by analyzing NS on six different days with triplicate measurements.

2.6. Data analysis

The data were expressed as means ± SD from three determinations. SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Significance of difference was tested at $P = 0.05$ by using ANOVA and Post Hoc Test (LSD). A reliability analysis was carried out by using intra-class correlation coefficients with a two-way mixed effects model.

3. Results and discussion

3.1. XRD pattern of chickpea starch samples

The RC of NS, RSI, RSII and RSIII were determined by XRD. Fig. 1 shows the XRD patterns of chickpea starch samples, and the RC of NS, RSI, RSII and RSIII were calculated to be 31.0 ± 1.1, 48.2 ± 0.9, 56.2 ± 3.4 and 52.5 ± 0.9%, respectively. Among them, the RC of NS was within the range in the literatures (27.6–34.4%) (Hughes et al.,